

SEAWATER CHALLENGE TESTS
ON SPRING CHINOOK
(Oncorhynchus tshawytscha)
AT THE
QUILCENE NATIONAL FISH HATCHERY

by

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U.S. Fish & Wildlife Service
Fisheries Assistance Office
Olympia, Washington

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Introduction

A principal goal of the U.S. Fish and Wildlife Service in Puget Sound and Coastal Washington is the restoration of depressed stocks of salmon and steelhead to levels which can sustain directed harvest. As part of this restoration effort, a brood run of spring chinook salmon is being developed in Hood Canal at the Quilcene National Fish Hatchery (NFH). Maximum survival of smolts to adult return is essential for the program's success. Past research indicates an increased survival for hatchery reared spring chinook released as yearlings. Based upon this research, we continue to recommend a yearling release strategy for the spring chinook salmon (SCS) program at Quilcene NFH. In addition to the length of rearing, however, there are a number of morphological, behavioral and physiological changes associated with the transformation from parr to smolt that are essential for marine survival (Folmar and Dickoff 1980). Wedemeyer et al., (1980) lists changes in 15 parameters of the smolt transformation process that must be properly coordinated. Knowledge of the timing of these changes on the Quilcene stock should aid in the determination of smolt functionality and the identification of a release "window" or optimum release time span. To date, only morphological observations and gill ($\text{Na}^+ - \text{K}^+$) ATPase levels are monitored on the Quilcene stock.

This study was undertaken to increase our knowledge of the smolt transformation process in the Quilcene spring chinook yearlings by examining additional parameters. The combination of seawater challenge survival and blood plasma sodium tests were recommended by Wedemeyer et al., (1980) and Clarke (1982) as a method of determining smolt functionality. The three parameters monitored in this study were: 1) the ability of chinook yearlings to survive the transition to seawater, 2) their ability to hypoosmoregulate (regulate their blood plasma level) during a seawater challenge test and 3) gill ($\text{Na}^+ - \text{K}^+$) ATPase levels. Fully smolted yearlings are expected to have the ability to survive the transition to seawater. Additionally, Clarke and Blackburn (1977) reported that fully smolted fish have the ability to maintain blood plasma levels at 170.0 meq/l or less within 24 hours after entry into seawater. Furthermore, increases in gill ATPase levels are associated with smoltification (Zaug and McLain, 1972), although critical levels of gill ATPase are not well known. Consequently, we monitored trends in gill ATPase activity in an attempt to relate peak activity to successful seawater challenge tests. These three criteria were compared in this study as a method of identifying functional smolts.

Methods and Materials

All seawater challenge tests were conducted at Quilcene NFH according to the techniques of Clarke and Blackburn (1977). The yearlings used in the tests were randomly selected from the 1984 brood of Quilcene spring chinook. The fish were reared outside under standard hatchery conditions in an 8 X 80 raceway until May 14, when the production lot was released. Since an effort was made to continue monitoring smoltification patterns beyond the current recommended release date, approximately 500 fish were removed from the 8X80 raceway on May 13 and transferred inside to a rearing tank. It was necessary to transfer the fish inside because the 8X80 raceways were needed to accommodate other groups of fish. This transfer resulted in a change in their water supply and rearing environment. The inside tanks are supplied only with water from Penny Creek. The outside raceways received, on the average, a combination of approximately 80% Quilcene River water and 20% Penny Creek water. A small amount of well water is also added to the outside raceways. Table 1 contains a description of the water composition for each test date.

TABLE 1. WATER COMPOSITION, TEMPERATURE AND SALINITY (PPT) OF WATER USED IN THE SEAWATER CHALLENGE TESTS.^{1/}

<u>DATE</u>	<u>WATER SOURCE</u>	<u>AVE. WATER TEMP. F</u>	<u>SALINITY</u>
4/15	Penny Creek (18.5%) Quilcene R. (75.7%) Well Water (5.8%)	45.5	34
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5/28	Penny Creek (100%)	50.1	25
6/12	Penny Creek (100%)	50.1	26-28
6/23	Penny Creek (100%)	56.0	26

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Based on the daily records of hatchery water use at Quilcene National Fish Hatchery : March - June 1986.

On each of the six test dates, 40 fish were randomly collected from the raceways. Ten fish were placed in each of four, 30 gallon plastic garbage cans that had been filled with water from the rearing unit. Two of the cans (controls) contained 25 gallons of freshwater taken directly from the rearing unit. The other two cans (test) contained 25 gallons of freshwater from the rearing unit mixed with varying amounts of California Aquarium Supply House-Synthetic Sea Water Mineral Concentrate to provide a saline environment. All four cans were partially submerged in the raceway or tank to maintain a water temperature similar to the rearing unit. All cans were aerated with Hagen Optima Air Pumps.

Initially, the seawater was mixed according to the manufacturer's recommendations to a full strength seawater environment (one bag of concentrate per 50 gallons of water). This resulted in a salinity of 34 parts per thousand (ppt) and very high mortality rates. After the second test, we decided to lower the amount of concentrate to provide a salinity of approximately 27 ppt., since we might not have had a sufficient number of challenged (test) survivors to measure blood sodium and evaluate hypoosmoregulatory ability. For the remaining four tests, the salinity ranged between 25-28 ppt., measured with an Atago hand refractometer. All fish were held in the cans for 24 hours as recommended by Clarke and Blackburn (1977).

After 24 hours, the fish were removed from the raceway cans but held in consistent water (fresh or salt) in the lab so that the specimens could be studied fresh. Groups of three or four live specimens were anesthetized with MS-222. Gross observations were made for evidence of hemorrhaging around the fin bases, dehydration, loss of parr marks and silvering on all specimens. Total length (TL) and weight (g) was also recorded so that condition factor (K-factor) could later be calculated. In addition, blood samples were taken from the live specimens. Blood was collected by removing the caudal region just posterior to the adipose fin, exposing the caudal aorta from which the sample was collected in a heparinized micro-hematocrit tube (Caraway capillary tube). A minimum of 10 microliters of plasma was necessary for analysis. After centrifuging for five minutes (Clay Adams-Serofuge centrifuge), the tube was cut to separate the plasma from the cells, and sealed to prevent loss and/or evaporation. Plasma samples were saved and taken to the Marrowstone Field Station, National Fisheries Research Center, Nordland, Washington, for analysis of blood sodium.

To measure the gill ATPase levels, additional groups of 10 fish were randomly collected by the hatchery staff from the raceway or tank, wrapped in freeze packs and shipped to Wally Zaugg, National Marine Fisheries Service, Cook Field Station Washington.

Because of the potential effects of disease on the seawater challenge test, we attempted to assess the pathological status of the fish. Pathogens, such as bacterial kidney disease (BKD), furunculosis or enteric red mouth could contribute to mortality

during the seawater challenge tests. At the onset of the project, kidney tissue samples from all live and dead specimens were aseptically obtained for analysis. Tissue smear and impression slides were made for the purpose of microscopic examinations using gram stain and fluorescent antibody techniques. Tissue streaks on trypticase soy agar (TSA) slants were made for the purpose of biochemical analysis. Fish kidneys were examined for the presence of gross lesions and observations of general fish condition were made. Both the microscopic examinations and the biochemical tests were discontinued after the second seawater challenge and only observations regarding gross lesions and general condition of the fish were made to assess fish health.

Results and Discussion

Seawater Survival

Seawater survival was the first criterion observed. A summary of seawater challenge performance is presented in Table 2. In the first two tests, when the salinity was 34 ppt, only 3 of 20 and 2 of 20 challenged yearlings survived, respectively. Apparently, the yearlings were not able to hypoosmoregulate in full strength seawater (34 ppt) in April. The 13 May test was the first test in which the salinity was lowered. The salinity on this date ranged from 25-27 ppt., but only 13 of 20 fish, or 65%, survived the 24 hour seawater challenge.

The last three tests had very high survival rates. On 12 June, 19 of the 20 survived, with the salinity ranging between 26-28 ppt. On 28 May and 23 June, 100% of the fish survived at salinities of 25 and 26 ppt, respectively. One unexplained mortality occurred among the controls (FW) on 28 May. No other control (FW) mortalities occurred. In terms of salinity tolerance, late May and June appear to be ideal release times.

It is impossible to know how much of an influence the increase in water temperature from 45.5 F to 50.1 F and the change in water source (Table 1) had on these results. We suspect that the change in rearing water may have imparted some bias in our results. In the past, increasing the contribution of Penny Creek relative to Quilcene River at the hatchery appeared to have beneficial effects on fish health and appeared to lower mortalities (Bill Thorson, personal communication). This therapeutic effect of the Penny Creek water source is attributed to its higher sodium and chloride ion concentration (Gatley, 1983, FWS Report).

Blood Sodium

In identifying a release "window" for the second criterion, blood sodium, results were analyzed using Clarke and Blackburn's (1977) criterion of 170.0 meq/l or less for fully smolted fish. A summary of the means for the control (FW) and test (SW) blood sodium levels are listed with confidence intervals in Table 3.

No discernible pattern was noted in the control blood sodium means. Throughout this study all of the control means were below 170.0, varying from a high of 166.8 on 23 June to a low of 144.3 on 12 June. However, the blood sodium values for fish held in saltwater exhibited considerably more variation (Figure 1). Mean blood sodium levels in these fish after 24 hours in saltwater were high initially, declined to a low in late May and then began to increase until testing terminated in late June. The highest SW mean was 235.5 on 28 April. This is, however, a mean of the only two saltwater survivors. The first test on 15 April also had a high mean of 205.0. Interestingly, one of the three challenged survivors passed the test with a level of 158.0.

Since the production lot was released on 14 May, we would have hoped to find the lowest mean blood sodium level on or near that date in the challenged fish. However, according to our tests on 14 May, the fish's mean blood sodium level was at 175.3. Only four of the tested 12 fish had blood sodium levels at 170.0 or below, even with the lowered salinity of 25-27 ppt. After the change to the Penny Creek water source on 28 May the group's mean blood sodium level fell below 170.0. All 20 challenged fish exhibited the ability to hypoosmoregulate on this day with a mean blood sodium level of 159.8.

On 12 June, the fish were still exhibiting satisfactory hypoosmoregulating ability, represented by the mean of 161.3 with 16 of 19 challenged fish or 84% passing the test. Again, late May and early June appear to be ideal release times as indicated by the results of this test. By 23 June, blood sodium levels had risen to 196.9. Smolted fish which are denied access to seawater are known to lose their hypoosmoregulating ability (Wedemeyer, 1980). However, the mean of 196.9 on 23 June is surprising, especially since all 20 fish survived the 24 hour saltwater exposure. Due to other demands at the Marrowstone Lab, the samples collected on this date were held in the refrigerator for one week. All other blood samples were analyzed on the same day. It is possible that there could have been some plasma evaporation which would have resulted in a higher concentration of sodium. However, the tubes were sealed and plugged, reducing chances of evaporation. Therefore, we suspect that the fish had begun to lose their ability to hypoosmoregulate.

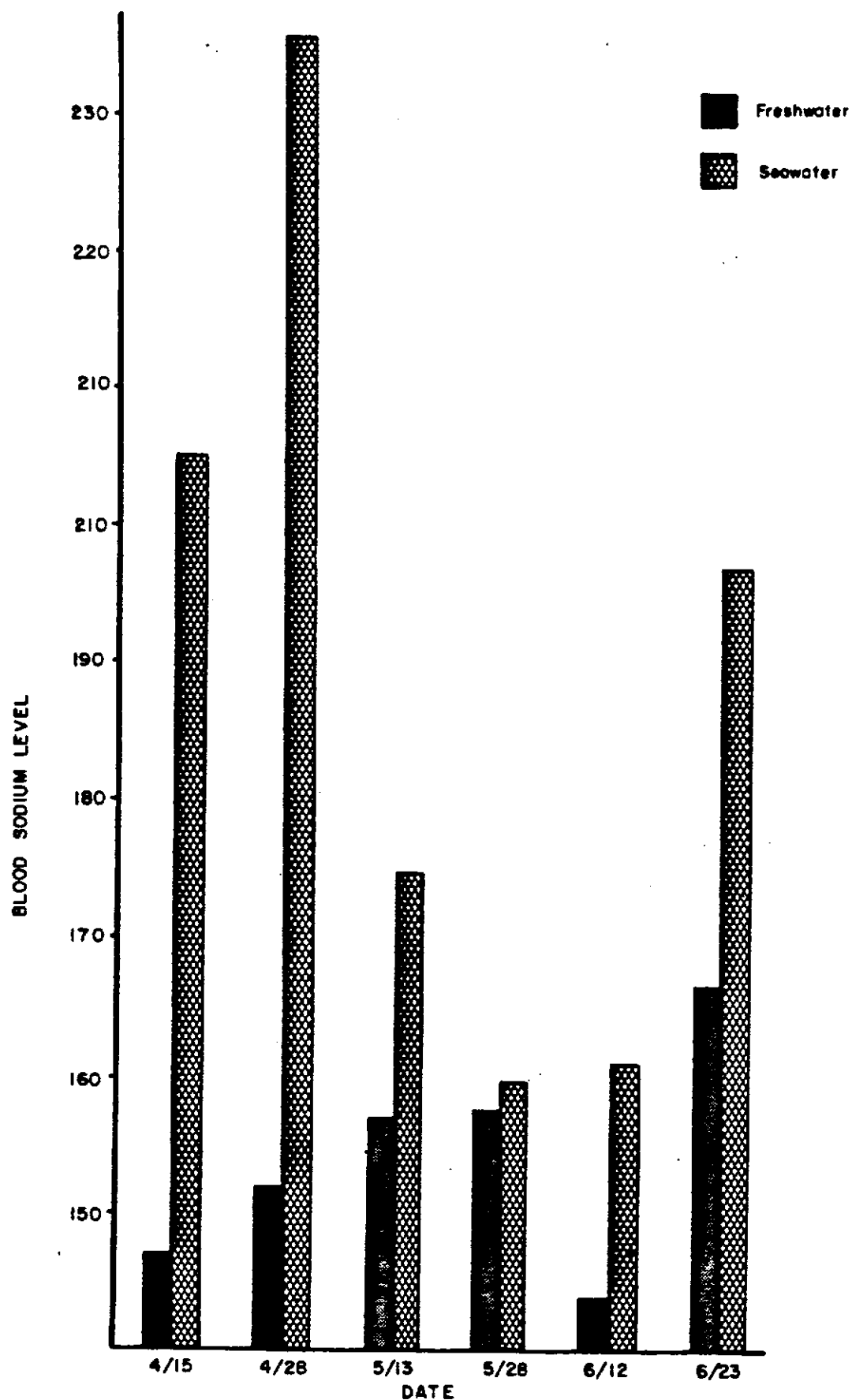


FIGURE 1. MEAN BLOOD SODIUM LEVELS (meq/L) OF FRESHWATER CONTROL AND SEAWATER CHALLENGED BROOD YEAR 1984 SPRING CHINOOK AT QUILCENE N.F.H., 1986

Gill (Na⁺ - K⁺) ATPase

The third and final criterion used in identifying an optimum release time was a peak in gill ATPase levels (Table 4). We were able to begin this portion of the test in mid-March, when ATPase was low 8.4 (μ moles Pi/mg protein hour). A short rise was reported in late March to 10.7 and again in mid-April to 13.2. Through April and mid-May, ATPase activity leveled off at approximately 13.0. Considerable rises were noted on 28 May to 19.9 and to 23.8 on 12 June. On the last test date, 23 June, ATPase was 23.3. Since tests ended on this date it is not possible to know if the ATPase level would have continued to rise. However, our figures indicate a leveling off after the peak on 12 June. Late May through June resulted in the highest likelihood of smoltification as represented by the ATPase activity pattern (Figure 2).

A decrease in condition factor is also known to be associated with the transition from parr to smolt (Wedemeyer et al., 1980). We assumed that the control fish represent the population and that the decreasing trend in condition factor noted during the first four tests is indicative of smolt transformation (Figure 3). On the date that we observed our lowest condition factor, 28 May, we also observed the best seawater survival and hypoosmoregulatory ability in the challenged fish.

Throughout this experiment, we assumed that the standard hatchery practices would not impart an effect on smolt development. Bacterial kidney disease (BKD), however, is known to be a chronic problem in spring chinook reared at Quilcene NFH and is believed to affect smolt development. The Olympia Fish Health Center determined that an increase in pre-release mortality in the 1984 brood was attributable to BKD. During April when our tests began, the hatchery experienced a 1.2% loss. This is an increase over the March loss of .91%. Additionally, they reported that the fish were emaciated and infected with Costia. The hatchery staff treated the fish for Costia on 28 April. Our examination of tissue samples during the first two tests indicated that only a small number of fish were infected with a low level of BKD. We made no attempt to record which fish were infected. Although we do not know the exact impact of BKD on the fish, it was determined that all mortalities which occurred during the seawater challenge were not directly attributable to BKD. No other reportable pathogens were detected during the time we examined tissue samples.

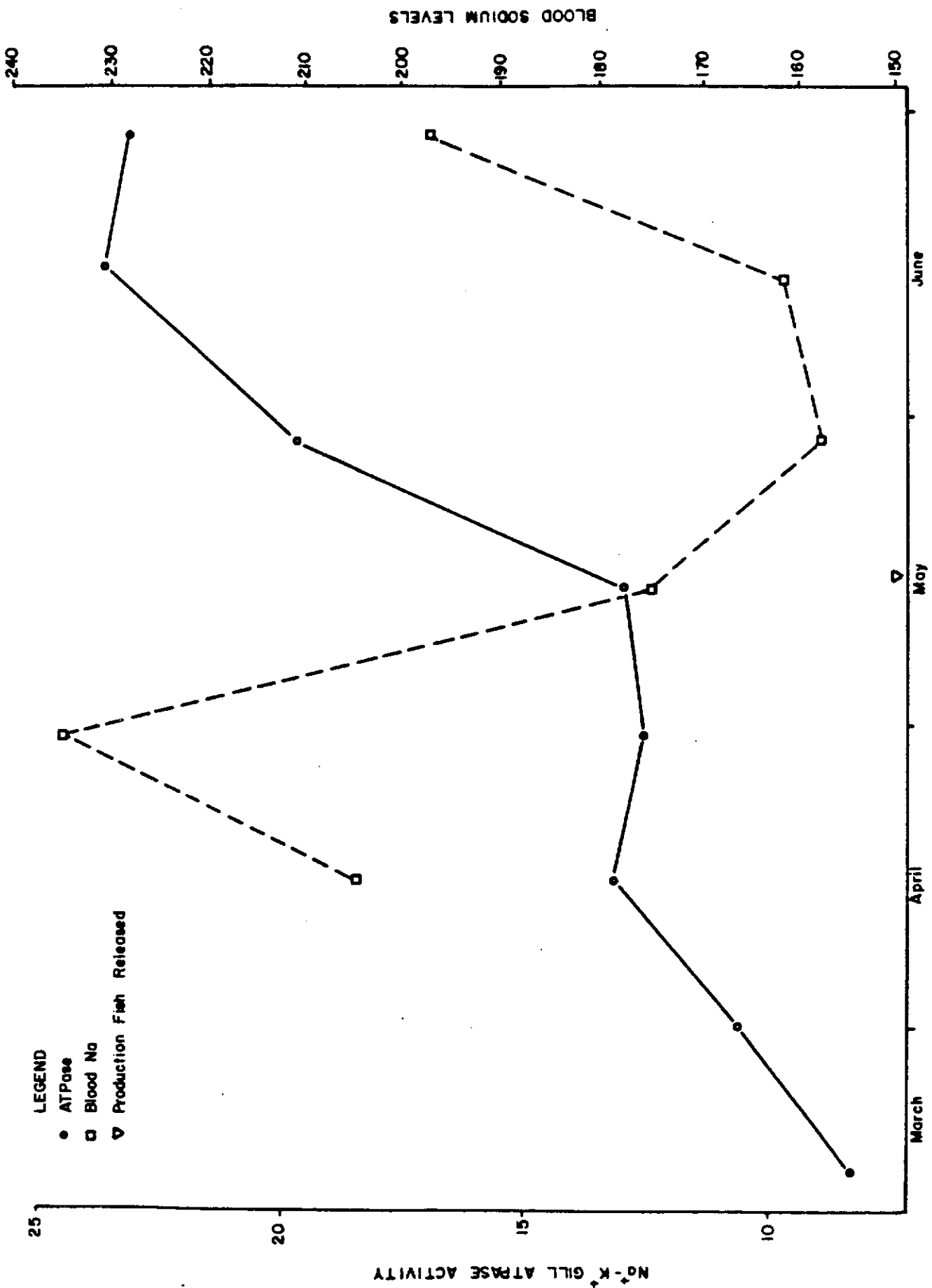


FIGURE 2. Na⁺-K⁺ ATPASE ACTIVITY (μmole Pi/mg protein·hour) AND SEAWATER CHALLENGE BLOOD SODIUM LEVELS (meq/L) OBSERVED IN BROOD YEAR 1984 SPRING CHINOOK REARED AT QUILCENE N.F.H., 1986.

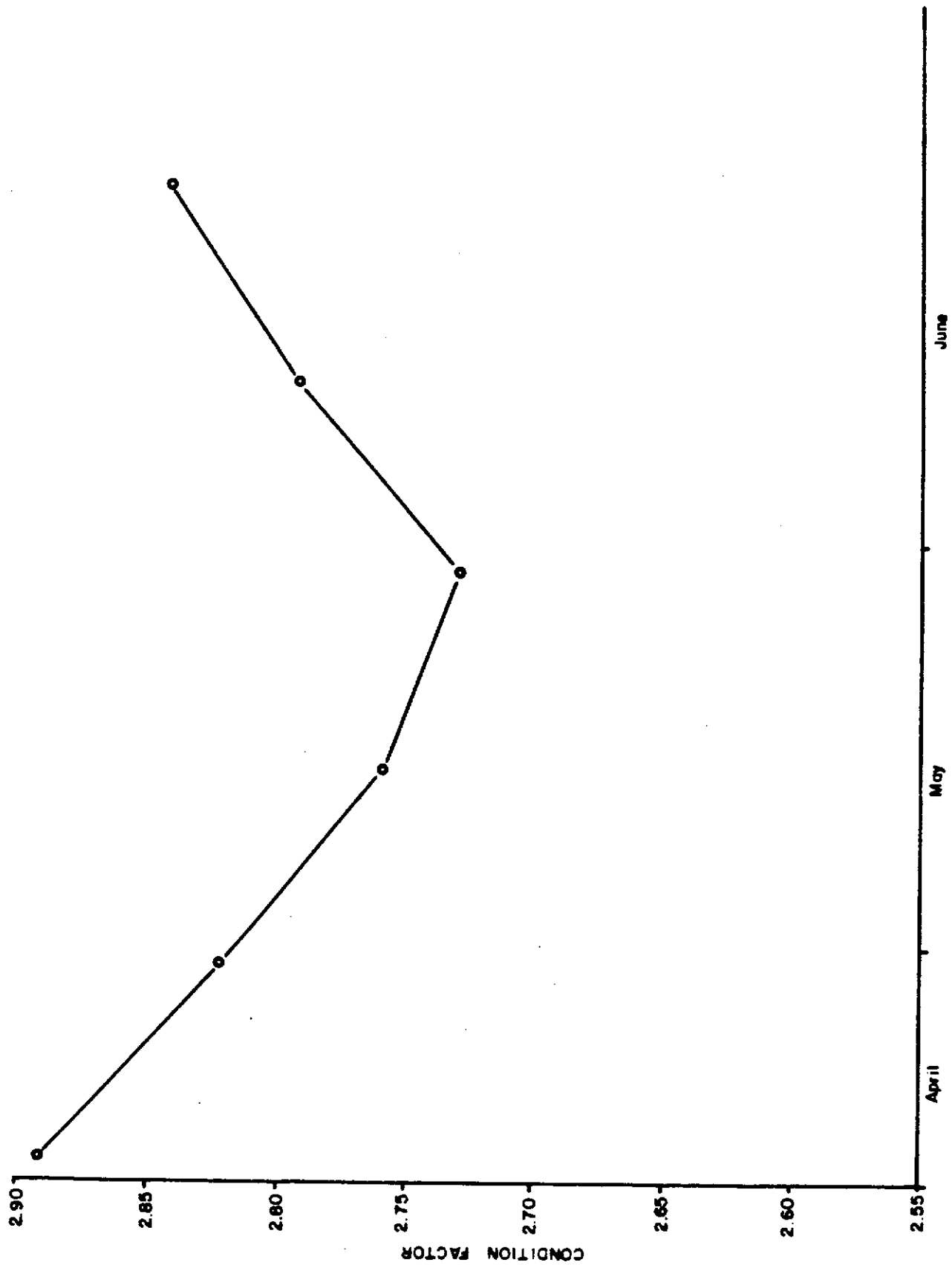


FIGURE 3. CONDITION FACTOR OBSERVED IN BROOD YEAR 1984 SPRING CHINOOK REARED AT QUILCENE N.F.H., 1986

Conclusions and Recommendations

A cursory analysis of our results would seem to indicate that the late May early June time span would be the optimum release time (Table 5). On 28 May, we experienced a 100% seawater survival rate, all fish were able to hypoosmoregulate their blood sodium levels below 170.0 and ATPase levels had risen substantially. On 12 June, the ATPase levels reached a peak, the survival rate continued to remain high (95%), and 84% of the challenged fish had the ability to regulate their blood sodium levels below 170.0 (Table 2). The results of this analysis should be viewed with caution, however, since the salinity of the challenge tests was altered, the fish were moved indoors, and maintained on a different water sources.

Despite these problems, we recommend that the seawater challenge blood sodium tests be repeated at Quilcene NFH. Once the techniques and methods are standardized, this test should aid in our understanding of a functional smolt in the Quilcene stock and in identifying an optimum release time period. To ensure consistency in the data, all test fish should be reared in a constant water supply and receive similar handling. An agreement needs to be developed between Olympia FAO and Quilcene NFH to insure a minimal impact on the seawater challenge tests without affecting hatchery production. We also recommend an initial examination of fish health to identify the pathological status of the stock since disease may impart an influence on test results. A concerted effort should be made to have all blood sodium analyzed within 24 hours of collection. Tentatively, we recommend the use of a 27 ppt salinity, but this needs to be researched further. All seawater challenge blood sodium tests and ATPase analysis should begin in early April and continue at least until the end of June.

TABLE 2. SUMMARY OF SEAWATER CHALLENGE PERFORMANCE AT QUILCENE NFH, 1986.

<u>DATE</u>	<u>NO. FISH CHALLENGED</u>	<u>NO. FISH SURVIVED</u>	<u>BLOOD SODIUM</u>		<u>% OF POP. EST. TO PASS</u>
			<u>NO. SAMPLED</u>	<u>NO. PASSED 1/</u>	
4/13	20	3	3	1	5
4/28	20	2	2	0	0
5/13	20	13	12	4	20
5/28	20	20	20	20	100
6/12	20	19	19	16	80
6/23	20	20	18	0	0

1/ Surviving fish with blood sodium levels below or equal to 170 meq/l.

TABLE 3. SUMMARY OF MEANS AND CONFIDENCE INTERVALS OF BLOOD SODIUM LEVELS AND CONDITION FACTOR.

<u>DATE</u>	<u>N</u>	<u>BLOOD NA 1/</u>		<u>N</u>	<u>CONDITION-FACTOR 2/</u>	
		<u>FW</u>			<u>FW</u>	
4/14	14	147.57 +	8.67	20	2.893 +	.086
4/28	13	152.10 +	3.05	20	2.824 +	.077
5/13	18	157.00 +	1.90	20	2.759 +	.098
5/28	19	157.70 +	1.99	19	2.729 +	.088
6/12	18	144.30 +	1.76	18	2.787 +	.084
6/23	20	166.80 +	5.80	20	2.84 +	.119

SW

4/14	3	205.0 +	106.54
4/28	2	235.5 +	133.41
5/13	12	175.3 +	11.36
5/28	20	159.8 +	1.86
6/12	19	161.3 +	6.52
6/23	18	196.9 +	6.47

TABLE 3. (continued)

1/Blood sodium figures include only live specimens
yielding blood samples (meq/l)

2/Condition factors include all live specimens

$$K = L / WT \times 36.12729 \times 10,000$$

K = condition factor

L = length (mm)

WT = weight (g)

TABLE 4. SUMMARY OF MEANS AND CONFIDENCE INTERVALS OF GILL ATPASE LEVELS.

<u>DATE</u>	<u>N</u>	<u>ATPase 1/</u>
3/17	10	8.40 + .78
3/31	9	10.68 + .52
4/14	10	13.20 + 2.21
4/28	10	12.60 + 2.55
5/12	10	13.00 + 3.12
5/28	9	19.90 + 4.08
6/12	9	23.80 + 2.61
6/23	9	23.28 + 5.44

1/ ATPase figures do not include precocious males
(moles Pi/mg protein hour).

TABLE 5. SUMMARY OF SMOLTIFICATION CRITERIA OBSERVED INCLUDING TOTAL SURVIVAL RATE, MEAN BLOOD SODIUM LEVELS AND MEAN ATPASE LEVELS.

<u>DATE</u>	<u>SURVIVAL</u>		<u>BLOOD SODIUM</u>		<u>ATPase</u>	
	<u>N</u>	<u>%</u>	<u>N</u>	<u>MEAN</u>	<u>N</u>	<u>MEAN</u>
April 15	3	15	3	205.5	10	13.2
April 28	2	10	2	235.5	10	12.6
May 13	13	65	12	175.3	10	13.0
May 28	20	100	20	159.8	9	19.9
June 12	19	95	19	161.3	9	23.8
June 23	20	100	18	196.9	9	23.3

ATPase = μ moles Pi/mg protein hour

Blood sodium = meq/l

Acknowledgments

We are most grateful to the Quilcene National Fish Hatchery staff for their cooperation and dedication to this project. Special thanks are extended to Bill Thorson and Ken Sexton for their technical assistance in conducting the seawater challenge tests and collecting blood samples.

We are indebted to Liz Carr for her assistance in the initial stages of the project as well as for the long hours spent analyzing fish health.

A special thank you is extended to Aldo Palmisano and Anita Cook of the National Fisheries Center, Marrowstone Field Station for blood sodium analyses.

Special thanks are also extended to Wally Zaugg of the National Fisheries Service, Cook Field Station for ATPase analyses.

We sincerely thank the Olympia Fish Health Lab for the lend of equipment, technical assistance and the biochemical analyses that they conducted.

Last but certainly not least, we thank all of the Olympia Fisheries Assistance Office staff for their review and recommendations throughout the project and on this manuscript.

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Results and Discussion

Seawater Survival

Seawater survival was the first criterion observed. A summary of seawater challenge performance is presented in Table 2. In the first two tests, when the salinity was 34 ppt, only 3 of 20 and 2 of 20 challenged yearlings survived, respectively. Apparently, the yearlings were not able to hypoosmoregulate in full strength seawater (34 ppt) in April. The 13 May test was the first test in which the salinity was lowered. The salinity on this date ranged from 25-27 ppt., but only 13 of 20 fish, or 65%, survived the 24 hour seawater challenge.

The last three tests had very high survival rates. On 12 June, 19 of the 20 survived, with the salinity ranging between 26-28 ppt. On 28 May and 23 June, 100% of the fish survived at salinities of 25 and 26 ppt, respectively. One unexplained mortality occurred among the controls (FW) on 28 May. No other control (FW) mortalities occurred. In terms of salinity tolerance, late May and June appear to be ideal release times.

It is impossible to know how much of an influence the increase in water temperature from 45.5 F to 50.1 F and the change in water source (Table 1) had on these results. We suspect that the change in rearing water may have imparted some bias in our results. In the past, increasing the contribution of Penny Creek relative to Quilcene River at the hatchery appeared to have beneficial effects on fish health and appeared to lower mortalities (Bill Thorson, personal communication). This therapeutic effect of the Penny Creek water source is attributed to its higher sodium and chloride ion concentration (Gatley, 1983, FWS Report).

Blood Sodium

In identifying a release "window" for the second criterion, blood sodium, results were analyzed using Clarke and Blackburn's (1977) criterion of 170.0 meq/l or less for fully smolted fish. A summary of the means for the control (FW) and test (SW) blood sodium levels are listed with confidence intervals in Table 3.

No discernible pattern was noted in the control blood sodium means. Throughout this study all of the control means were below 170.0, varying from a high of 166.8 on 23 June to a low of 144.3 on 12 June. However, the blood sodium values for fish held in saltwater exhibited considerably more variation (Figure 1). Mean blood sodium levels in these fish after 24 hours in saltwater were high initially, declined to a low in late May and then began to increase until testing terminated in late June. The highest SW mean was 235.5 on 28 April. This is, however, a mean of the only two saltwater survivors. The first test on 15 April also had a high mean of 205.0. Interestingly, one of the three challenged survivors passed the test with a level of 158.0.

Since the production lot was released on 14 May, we would have hoped to find the lowest mean blood sodium level on or near that date in the challenged fish. However, according to our tests on 14 May, the fish's mean blood sodium level was at 175.3. Only four of the tested 12 fish had blood sodium levels at 170.0 or below, even with the lowered salinity of 25-27 ppt. After the change to the Penny Creek water source on 28 May the group's mean blood sodium level fell below 170.0. All 20 challenged fish exhibited the ability to hypoosmoregulate on this day with a mean blood sodium level of 159.8.

On 12 June, the fish were still exhibiting satisfactory hypoosmoregulating ability, represented by the mean of 161.3 with 16 of 19 challenged fish or 84% passing the test. Again, late May and early June appear to be ideal release times as indicated by the results of this test. By 23 June, blood sodium levels had risen to 196.9. Smolted fish which are denied access to seawater are known to lose their hypoosmoregulating ability (Wedemeyer, 1980). However, the mean of 196.9 on 23 June is surprising, especially since all 20 fish survived the 24 hour saltwater exposure. Due to other demands at the Marrowstone Lab, the samples collected on this date were held in the refrigerator for one week. All other blood samples were analyzed on the same day. It is possible that there could have been some plasma evaporation which would have resulted in a higher concentration of sodium. However, the tubes were sealed and plugged, reducing chances of evaporation. Therefore, we suspect that the fish had begun to lose their ability to hypoosmoregulate.

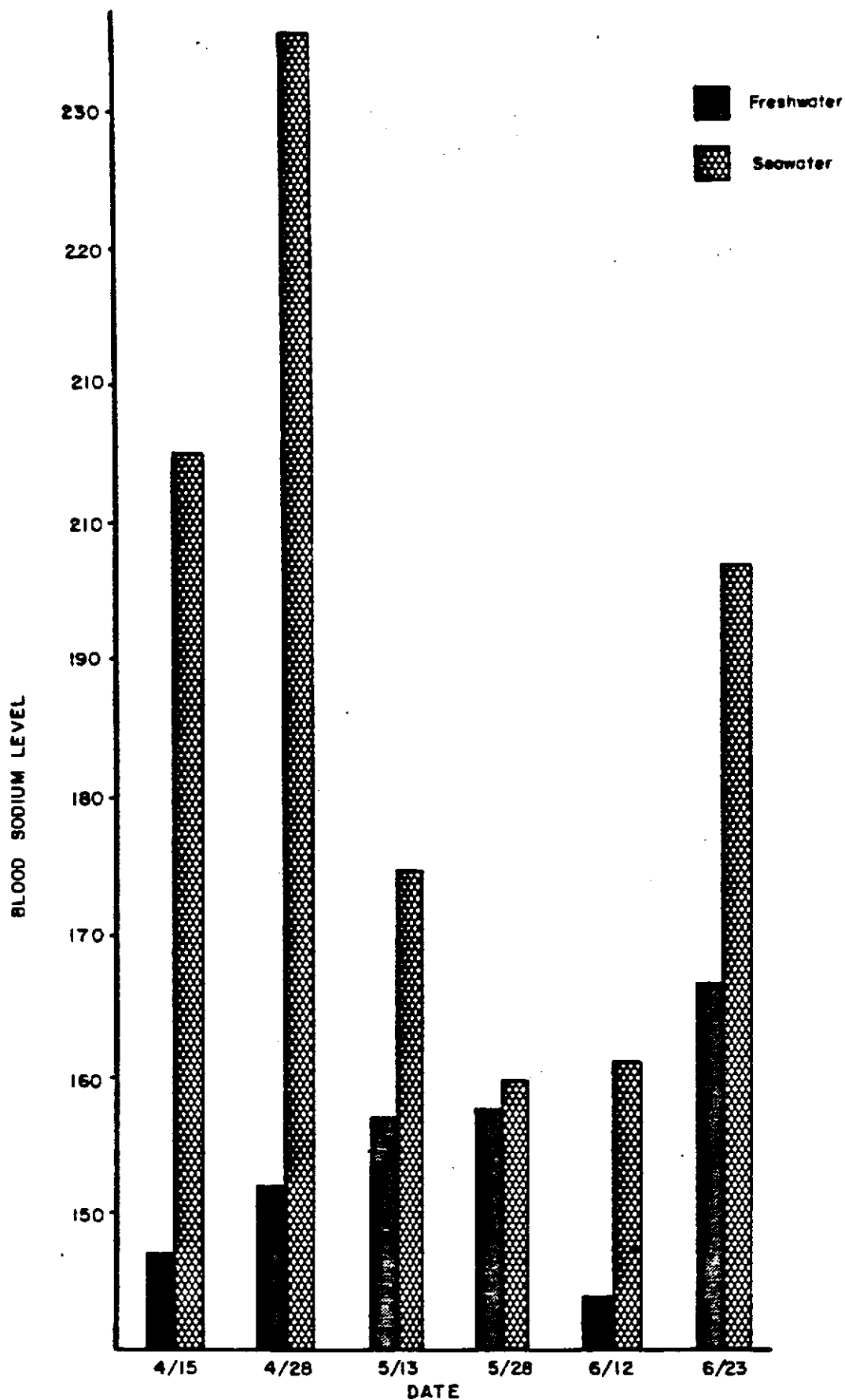


FIGURE 1. MEAN BLOOD SODIUM LEVELS (meq/L) OF FRESHWATER CONTROL AND SEAWATER CHALLENGED BROOD YEAR 1984 SPRING CHINOOK AT QUILCENE N.F.H., 1986

Gill (Na⁺ - K⁺) ATPase

The third and final criterion used in identifying an optimum release time was a peak in gill ATPase levels (Table 4). We were able to begin this portion of the test in mid-March, when ATPase was low 8.4 (μ moles Pi/mg protein hour). A short rise was reported in late March to 10.7 and again in mid-April to 13.2. Through April and mid-May, ATPase activity leveled off at approximately 13.0. Considerable rises were noted on 28 May to 19.9 and to 23.8 on 12 June. On the last test date, 23 June, ATPase was 23.3. Since tests ended on this date it is not possible to know if the ATPase level would have continued to rise. However, our figures indicate a leveling off after the peak on 12 June. Late May through June resulted in the highest likelihood of smoltification as represented by the ATPase activity pattern (Figure 2).

A decrease in condition factor is also known to be associated with the transition from parr to smolt (Wedemeyer et al., 1980). We assumed that the control fish represent the population and that the decreasing trend in condition factor noted during the first four tests is indicative of smolt transformation (Figure 3). On the date that we observed our lowest condition factor, 28 May, we also observed the best seawater survival and hypoosmoregulatory ability in the challenged fish.

Throughout this experiment, we assumed that the standard hatchery practices would not impart an effect on smolt development. Bacterial kidney disease (BKD), however, is known to be a chronic problem in spring chinook reared at Quilcene NFH and is believed to affect smolt development. The Olympia Fish Health Center determined that an increase in pre-release mortality in the 1984 brood was attributable to BKD. During April when our tests began, the hatchery experienced a 1.2% loss. This is an increase over the March loss of .91%. Additionally, they reported that the fish were emaciated and infected with Costia. The hatchery staff treated the fish for Costia on 28 April. Our examination of tissue samples during the first two tests indicated that only a small number of fish were infected with a low level of BKD. We made no attempt to record which fish were infected. Although we do not know the exact impact of BKD on the fish, it was determined that all mortalities which occurred during the seawater challenge were not directly attributable to BKD. No other reportable pathogens were detected during the time we examined tissue samples.

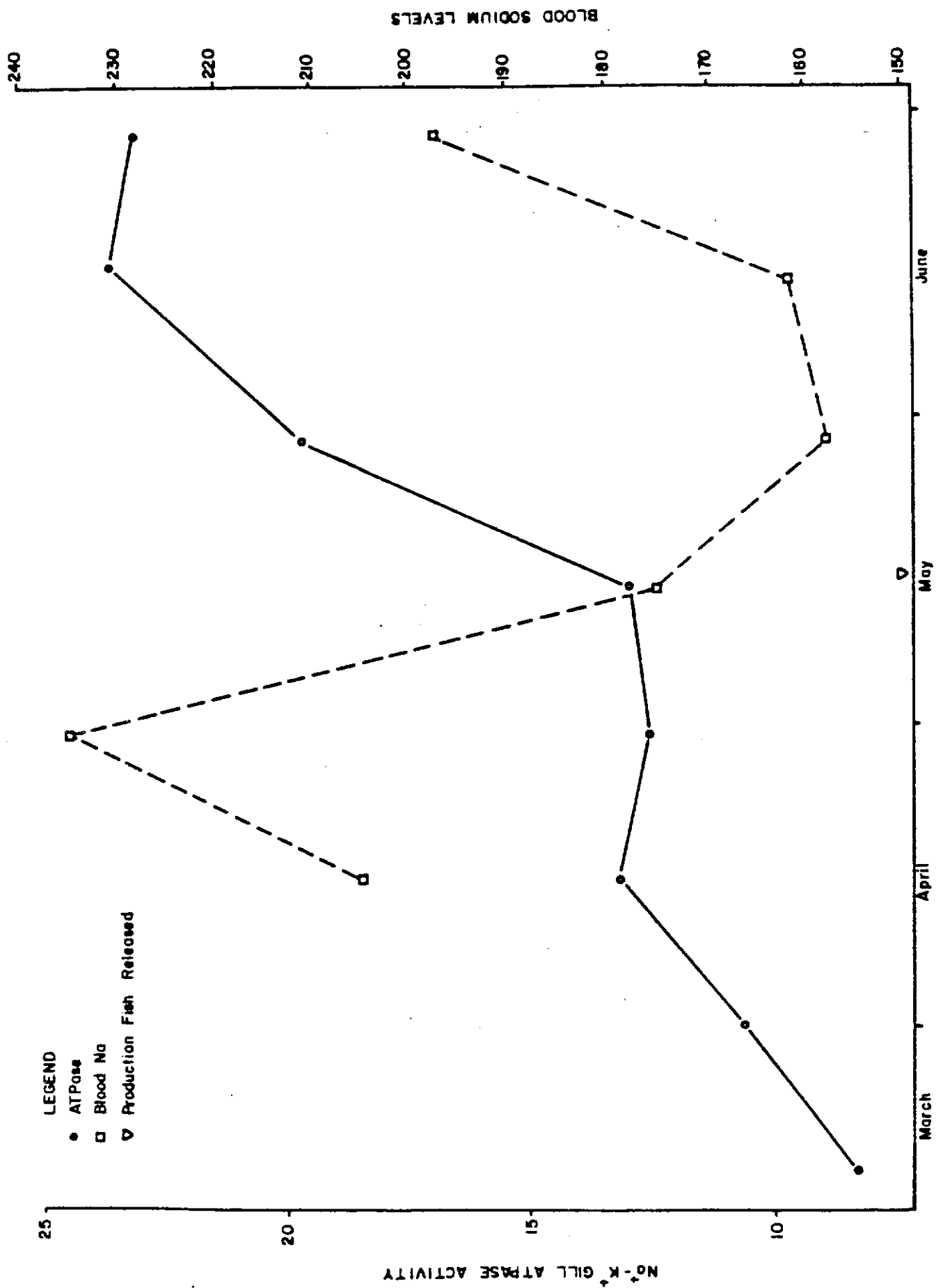


FIGURE 2. Na⁺-K⁺ ATPase activity (μmole Pi/mg protein·hour) and seawater challenge blood sodium levels (meq/L) observed in brood year 1984 spring chinook reared at Quilcène N.F.H., 1986.

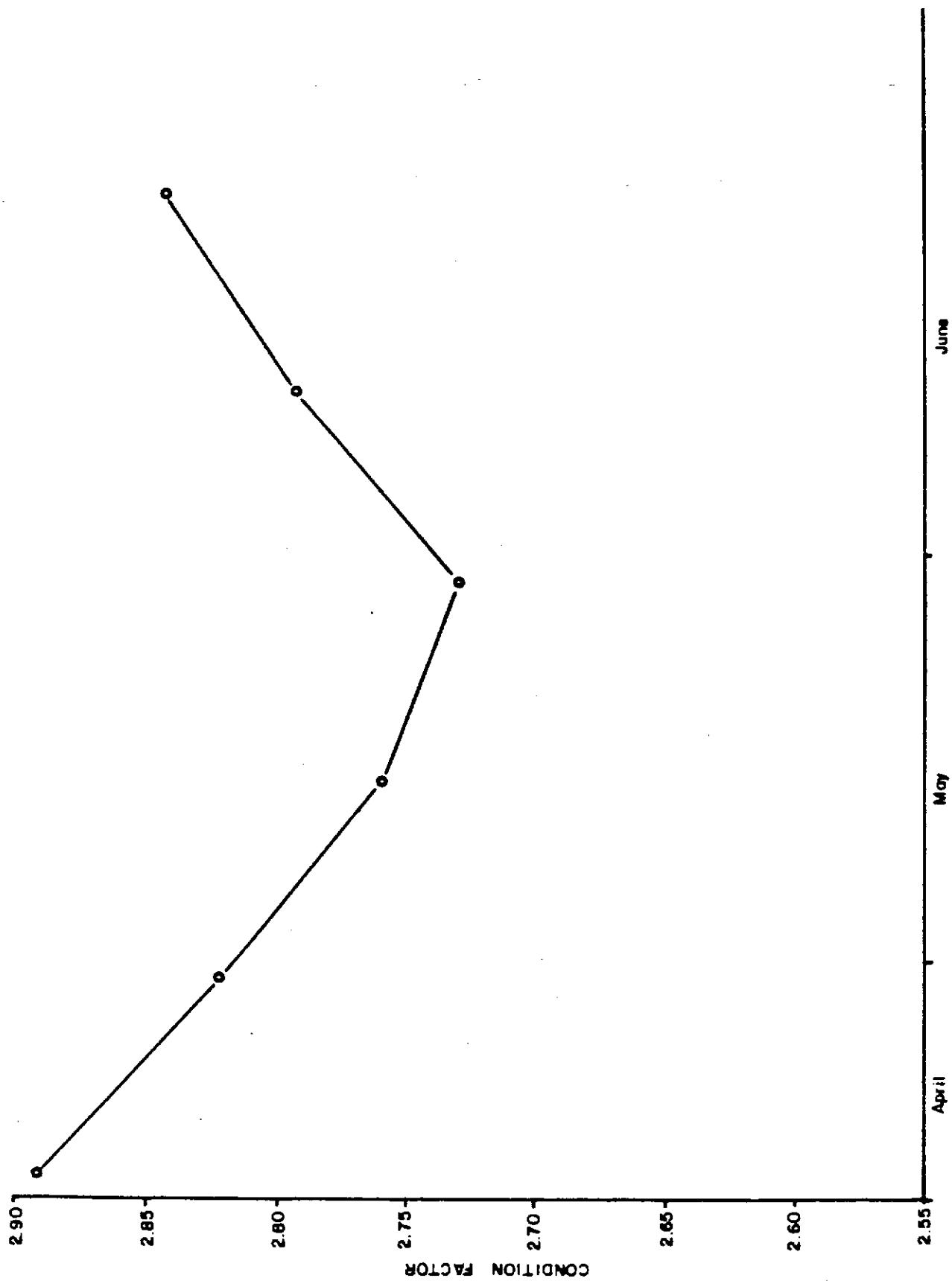


FIGURE 3. CONDITION FACTOR OBSERVED IN BROOD YEAR 1984 SPRING CHINOOK REARED AT QUILCENE N.F.H., 1986

Conclusions and Recommendations

A cursory analysis of our results would seem to indicate that the late May early June time span would be the optimum release time (Table 5). On 28 May, we experienced a 100% seawater survival rate, all fish were able to hypoosmoregulate their blood sodium levels below 170.0 and ATPase levels had risen substantially. On 12 June, the ATPase levels reached a peak, the survival rate continued to remain high (95%), and 84% of the challenged fish had the ability to regulate their blood sodium levels below 170.0 (Table 2). The results of this analysis should be viewed with caution, however, since the salinity of the challenge tests was altered, the fish were moved indoors, and maintained on a different water sources.

Despite these problems, we recommend that the seawater challenge blood sodium tests be repeated at Quilcene NFH. Once the techniques and methods are standardized, this test should aid in our understanding of a functional smolt in the Quilcene stock and in identifying an optimum release time period. To ensure consistency in the data, all test fish should be reared in a constant water supply and receive similar handling. An agreement needs to be developed between Olympia FAO and Quilcene NFH to insure a minimal impact on the seawater challenge tests without affecting hatchery production. We also recommend an initial examination of fish health to identify the pathological status of the stock since disease may impart an influence on test results. A concerted effort should be made to have all blood sodium analyzed within 24 hours of collection. Tentatively, we recommend the use of a 27 ppt salinity, but this needs to be researched further. All seawater challenge blood sodium tests and ATPase analysis should begin in early April and continue at least until the end of June.

TABLE 2. SUMMARY OF SEAWATER CHALLENGE PERFORMANCE AT QUILCENE NFH, 1986.

DATE	NO. FISH CHALLENGED	NO. FISH SURVIVED	BLOOD SODIUM		% OF POP. EST. TO PASS
			NO. SAMPLED	NO. PASSED 1/	
4/13	20	3	3	1	5
4/28	20	2	2	0	0
5/13	20	13	12	4	20
5/28	20	20	20	20	100
6/12	20	19	19	16	80
6/23	20	20	18	0	0

1/ Surviving fish with blood sodium levels below or equal to 170 meq/l.

TABLE 3. SUMMARY OF MEANS AND CONFIDENCE INTERVALS OF BLOOD SODIUM LEVELS AND CONDITION FACTOR.

DATE	N	BLOOD NA 1/		N	CONDITION-FACTOR 2/	
		FW			FW	
4/14	14	147.57	+ 8.67	20	2.893	+ .086
4/28	13	152.10	+ 3.05	20	2.824	+ .077
5/13	18	157.00	+ 1.90	20	2.759	+ .098
5/28	19	157.70	+ 1.99	19	2.729	+ .088
6/12	18	144.30	+ 1.76	18	2.787	+ .084
6/23	20	166.80	+ 5.80	20	2.84	+ .119

SW

4/14	3	205.0	+ 106.54
4/28	2	235.5	+ 133.41
5/13	12	175.3	+ 11.36
5/28	20	159.8	+ 1.86
6/12	19	161.3	+ 6.52
6/23	18	196.9	+ 6.47

TABLE 3. (continued)

1/Blood sodium figures include only live specimens yielding blood samples (meq/l)

2/Condition factors include all live specimens

$K = L / WT \times 36.12729 \times 10,000$

K = condition factor

L = length (mm)

WT = weight (g)

TABLE 4. SUMMARY OF MEANS AND CONFIDENCE INTERVALS OF GILL ATPASE LEVELS.

DATE	N	ATPase 1/
3/17	10	8.40 + .78
3/31	9	10.68 + .52
4/14	10	13.20 + 2.21
4/28	10	12.60 + 2.55
5/12	10	13.00 + 3.12
5/28	9	19.90 + 4.08
6/12	9	23.80 + 2.61
6/23	9	23.28 + 5.44

1/ ATPase figures do not include precocious males (moles Pi/mg protein hour).

TABLE 5. SUMMARY OF SMOLTIFICATION CRITERIA OBSERVED INCLUDING TOTAL SURVIVAL RATE, MEAN BLOOD SODIUM LEVELS AND MEAN ATPASE LEVELS.

DATE	SURVIVAL		BLOOD SODIUM		ATPase	
	N	%	N	MEAN	N	MEAN
April 15	3	15	3	205.5	10	13.2
April 28	2	10	2	235.5	10	12.6
May 13	13	65	12	175.3	10	13.0
May 28	20	100	20	159.8	9	19.9
June 12	19	95	19	161.3	9	23.8
June 23	20	100	18	196.9	9	23.3

ATPase = μ moles Pi/mg protein hour

Blood sodium = meq/l

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